

§ 1.136(a), and any fees required therefor (including fees for net addition of claims) are hereby authorized to be charged to our Deposit Account No. 19-0036.

*Amendments*

*In the Specification:*

Please substitute the following paragraphs in place of the pending paragraphs:

*At page 17, line 28 through page 18, line 17*

CI  
About 80,000 cells were seeded into a 6 cm well in a 6-well culture dish and cultivated in DMEM, supplemented with 10 % FCS (v/v), 2 mM glutamine, 100 International Units of penicillin and 100 µg/ml of streptomycin, until 70 % confluence was achieved. The cells were transfected with 1 µg of a plasmid coding for eGFP ("enhanced GFP"; pEGFP-C1; Clontech) together with 1 µg of a control plasmid (pMEX; (22)), a pCMV plasmid containing the pro-apoptotic Adenovirus gene E1A or a pCMV plasmid containing the anti-apoptotic Adenovirus gene E1B-19K (17, 18) using 10 µl of LipofectAMINE™ (GIBCO-BRL) in accordance with the manufacturer's recommendations. After the transfection the cells were left to stand for 16 h in complete medium, then the cells were either left untreated or treated for a further 16 h with an apoptotic stimulus (800 ng/ml of staurosporin; Sigma) (19, 20). 32 h after the transfection the detached cells were combined with trypsinised, adherent cells, washed twice with 4 ml of PBS and fixed at ambient temperature for 30 min (2% paraformaldehyde, 100 mM NaCl, 300 mM saccharose, 3 mM MgCl<sub>2</sub>, 1 mM EGTA

C1  
4 (ethyleneglycol-bis-(2-aminoethyl)-tetraacetic acid), 10 mM PIPES (piperazine-N<sub>1</sub>N<sup>1</sup>-bis[z-ethanesulphonic acid]) pH 6.8). Then they were washed twice with 4 ml of PBS and fixed for 14 h in ice-cold 70 % EtOH.

---

*At page 18, line 19 through page 19, line 29*

---

C2 After the fixing, the cells were washed twice with 4 ml of PBS and divided up. One half of the sample was treated with RNase A (Sigma, St. Louis, USA) (50 µg/ml) in PBS for 30 min, washed twice with 4 ml of PBS and, 30 min before the FACS analysis, stained with propidium iodide in PBS (PI; 50 µg/ml; Sigma, St. Louis, USA). The other half of the sample was incubated for 1 hour at 37° C with 50 µl of TdT reaction mixture (terminal deoxynucleotidyl transferase; Boehringer Mannheim; 200 mM potassium cacodylate, 25 mM of Tris-HCl, pH 6.6, 0.25 mg/ml bovine serum albumen, 1 mM CoCl<sub>2</sub>; 0.25 nmol FluoroLink™ Cy5AP3-dCTP [Amersham], 12.5 units of TdT), washed twice with 4 ml of PBS, treated with RNase in PBS (50 µg/ml) for 30 min, washed twice with 4 ml HBS (from this step onwards HBS was used instead of PBS because DAPI has a tendency to produce microprecipitates in PBS), stained with DAPI in HBS (10 µg/ml; Sigma) for 20 min and analysed using a Becton Dickinson FACSVantage™ apparatus. The FACS analysis of the PI-stained cells was carried out with a Becton Dickinson FACScan apparatus fitted with a so-called "doublet discrimination module", with which cell aggregates are discriminated by calculating the pulse width and pulse width. The results of the tests are shown in Fig.1. Fig. 1A shows the number of apoptotic βHC 13T tumour cells (% apoptosis) in the entire eGFP-positive cell population. The black bars indicate the determination of the sub-2N DNA

C2  
✓

content (GFP/PI); the white bars indicate the incorporation of fluorescent Cy5AP3-dCTP during the TdT reaction (GFP/TUNEL). The addition of staurosporin is shown. An excitation wavelength of 488 nm was used for eGFP and PI, an excitation wavelength of 647 nm was used for Cy5 and UV of a wavelength range of 51 - 364 nm was used for DAPI. The emission fluorescence was collected using a 530/20 nm narrow band filter for eGFP, a 610 nm blocking filter for PI, a 675/20 nm narrow band filter for Cy5 and a 424/44 narrow band filter for DAPI. Doublets were excluded by means of pulsed processing. eGFP-expressing cells were selected and analysed for Cy5- or PI-fluorescence. The data were analysed using CELLQuest™ software (Becton Dickinson). Each bar represents the average of 3 transfections, standard deviations are indicated by error bars. Each measurement comprised 40,000 total events, selected according to size and single cells. The transfection efficiency was 20-30 %.

---

*At page 20, lines 6-19*

---

C3

In this Example an untransformed rat fibroblast cell line designated Rat1A was used. The cells were transiently transfected using either LipofectAMINE™, as described in Example 1, or polyethyleneimine (PEI 2000)-DNA-Adenovirus complexes (WO 93/07283). Moreover, regarding the treatment of the cells and determination of apoptosis, using the process according to the invention on the one hand and the TUNEL method on the other hand, the procedure was exactly as described in Example 1. A comparison of the different transfection methods and methods of measuring apoptosis is shown in the Table. Each value represents the average of 3 transfections; the standard deviation is given (s.d.).

---